Benzene, 1-methyl-2,4-dinitro-: Human health tier II assessment

18 September 2014

CAS Number: 121-14-2

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted

and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

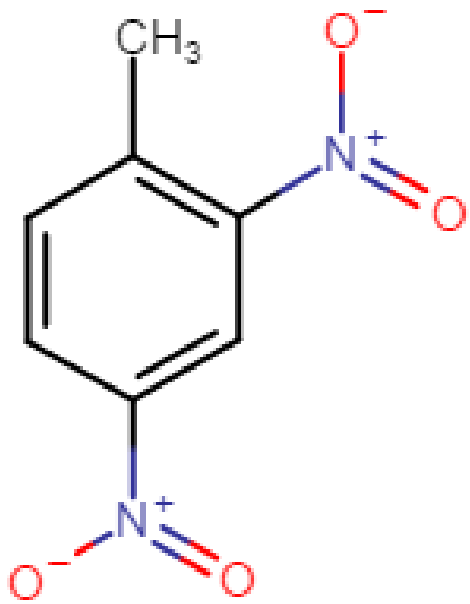
For more detail on this program please visit:www.nicnas.gov.au

Disclaimer

NICNAS has made every effort to assure the quality of information available in this report. However, before relying on it for a specific purpose, users should obtain advice relevant to their particular circumstances. This report has been prepared by NICNAS using a range of sources, including information from databases maintained by third parties, which include data supplied by industry. NICNAS has not verified and cannot guarantee the correctness of all information obtained from those databases. Reproduction or further distribution of this information may be subject to copyright protection. Use of this information without obtaining the permission from the owner(s) of the respective information might violate the rights of the owner. NICNAS does not take any responsibility whatsoever for any copyright or other infringements that may be caused by using this information.

Acronyms & Abbreviations

Chemical Identity

Synonyms	2,4-dinitrotoluene 2,4-DNT 4-methyl-1,3-dinitrobenzene
Structural Formula	
Molecular Formula	C7H6N2O4
Molecular Weight (g/mol)	182.1
Appearance and Odour (where available)	Yellow or orange crystals
SMILES	c1(C)c(N(=O)=O)cc(N(=O)=O)cc1

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified.

International

The following international uses have been identified through:

- the Organisation for Economic Cooperation and Development Screening information data set International Assessment Report (OECD SIAR);
- Galleria Chemica;
- the Substances and Preparations in the Nordic countries (SPIN) database;
- the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and
- various international assessments (OECD, 1996; EURAR, 2008; ACGIH, 2011; ATSDR, 2013).

The chemical has reported commercial use including:

- as a gelatinising, plasticising and waterproofing agent in both commercial and military explosive compositions;
- as a modifier for smokeless powders in the munitions industry; and
- in the airbags of motor vehicles.

The chemical has reported site-limited use including as a chemical intermediate in the production of:

- toluenediisocyanates, precursors to polyurethane polymers (major use);
- trinitrotoluene (TNT);
- dyes and dyestuffs; and
- rubber chemicals.

Restrictions

Australian

No known restrictions have been identified.

International

The chemical is listed on the following (Galleria Chemica):

- EU Cosmetics Regulation 1223/2009 Annex II—List of substances prohibited in cosmetic products;
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain; and

- Association of South East Asian Nations (ASEAN) Cosmetic Directive Annex II Part 1: List of substances which must not form part of the composition of cosmetic products.

The chemical is listed in Annex XIV "The Authorization List" with a sunset date of 21 August 2015 (date from which the placing on the market and the use of a substance is prohibited, unless an authorisation is granted) (ECHA, 2014).

Existing Work Health and Safety Controls

Hazard Classification

The chemical is classified as hazardous, with the following risk phrases for human health in the Hazardous Substances Information System (HSIS) (Safe Work Australia):

Carc. Cat. 2; R45 (carcinogenicity);

Muta. Cat. 3; R68 (mutagenicity);

Repr. Cat. 3; R62 (reproductive toxicity);

T; R23/24/25 (acute toxicity); and

Xn; R48/22 (repeated dose toxicity).

Exposure Standards

Australian

No specific exposure standards are available for 2,4-dinitrotoluene (2,4-DNT—CAS No. 121-14-2).

The related chemical technical grade (Tg) dinitrotoluene (DNT—CAS No. 25321-14-6), comprises mixed isomers of dinitrotoluene (up to six isomers are possible, although 2,4-DNT and 2,6-DNT normally dominate). This has an exposure standard of 1.5 mg/m^3 time weighted average (TWA). A skin notation (Sk—absorption through the skin may be a significant source of exposure) is assigned (Safe Work Australia).

Guidance on the interpretation of workplace exposure standards for airborne contaminants advises that 'exposure to carcinogens should be eliminated or minimised so far as is reasonably practicable' (Safe Work Australia, 2013).

International

The following exposure standards are identified (Galleria Chemica) for 2,4-DNT.

An exposure limit (occupational exposure limit (OEL), permissible exposure limit (PEL)) of 1 mg/m^3 (TWA) in different countries such as Russia and Latvia.

The following exposure standards are identified (Galleria Chemica) for the related chemical DNT, which covers mixed isomers of dinitrotoluene including 2,4-DNT:

An exposure limit (OEL, PEL) of 0.15–1.5 mg/m^3 (TWA) and 0.3–5 mg/m^3 short-term exposure limit (STEL) in different countries such as the USA, China, Canada (Yukon), Denmark, Norway, Mexico, Sweden and South Africa.

The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value (TLV) of 0.2 mg/m^3 (TWA) for dinitrotoluene (CAS No. 25321-14-6). This value is 'intended to minimise the potential for heart disease and possible reproductive effects. It should also provide protection against methemoglobinemia'. A skin notation is assigned (ACGIH, 2011).

Health Hazard Information

The chemical is a primary component of the dinitrotoluene (DNT) commercial mixture. DNT (CAS No. 25321-14-6) comprises approximately 76 % 2,4-DNT (CAS No. 121-14-2) and 20 % 2,6-DNT (CAS No. 606-20-2).

Toxicokinetics

The toxicokinetics of the chemical have been investigated in laboratory animals and in humans (EURAR, 2008; ACGIH, 2011; ATSDR, 2013).

The available animal data demonstrate that the chemical can be readily absorbed following oral exposure. Biological monitoring in workers exposed to technical grade dinitrotoluene indicated that dermal and inhalation (in the form of dusts and aerosols) exposure could be a significant route of entry for the chemical in humans.

Once absorbed, the chemical and its metabolites are distributed throughout the body with a similar pattern of distribution in all animal species. In a radiolabelling study, levels of radioactivity were typically higher in the liver and kidneys. There is insufficient evidence of accumulation in these organs.

The route of excretion was similar in all animal species. The majority of the chemical and its metabolites are excreted in the urine. The major urinary metabolites detected were the glucuronide conjugates of 2,4-dinitrobenzyl alcohol and 2,4-(amino,nitro)-benzyl alcohols (animal studies) and 2,4-dinitrobenzoic acid (humans). Bile is an important route of excretion. A larger percentage of the administered dose is excreted in the bile of male rats than in females.

The liver and intestinal microflora both appear to play an important role in the metabolism of the chemical. The chemical is initially oxidised in the liver by cytochrome P-450 to form 2,4-dinitrobenzyl alcohol. The glucuronide conjugate of this metabolite is eliminated via urine or excreted in bile. In the bile this metabolite is released into the gut where it is metabolised by intestinal microflora (by hydrolysis and reduction of the nitro group) to form aminonitrobenzyl alcohols. A portion of the aminonitrobenzyl alcohols are reabsorbed and transported back to the liver where they are metabolised into reactive species capable of binding to DNA (see **Genotoxicity)**. Data indicate that sulfation is important in the biotransformation of the chemical to reactive metabolites.

Acute Toxicity

Oral

The chemical is classified as hazardous with the risk phrase ‘Toxic if swallowed’ (T; R25) in HSIS (Safe Work Australia). The available reliable median lethal dose (LD50) values (434–743 mg/kg bw) in rats do not support this classification. However, methaemoglobinaemia was observed following a single exposure to relatively low doses. An amendment of the classification based on these non-lethal effects is recommended (refer **Recommendation** section).

In a study conducted similarly to test guidelines with the chemical (purity 98 %), the acute oral LD50 (95 % confidence limits) was:

- 568 (434–705) mg/kg bw and 650 (520–743) mg/kg bw in male and female CD rats, respectively; and
- 1954 (1848–2178) mg/kg bw and 1340 (1205–1500) mg/kg bw in male and female albino Swiss mice, respectively.

In another study, no mortality was observed in rats dosed with 375 mg/kg 2,4-DNT (97–99 % purity) by gavage (EU RAR, 2008).

Increased levels on both methaemoglobin and Heinz bodies were observed in cats following single exposure to 50 mg/kg bw (oral) and 20–40 mg/kg bw (intraperitoneally (i.p.)). No effects were observed following oral exposure to 10 mg/kg bw. Humans and cats are more sensitive to methaemoglobin formation than rodents (EU RAR, 2008).

Dermal

The chemical is classified as hazardous with the risk phrase 'Toxic in contact with skin' (T; R24) in HSIS (Safe Work Australia). The available LD50 values (>2500 mg/kg bw) do not support this classification (EU RAR, 2008). An amendment to the classification is recommended (refer **Recommendation** section) based on the following:

- observed methaemoglobinaemia following single exposure (oral and i.p.) to relatively low doses of the chemical (see Acute toxicity:oral); and
- skin absorption is significant source of exposure (ACGIH, 2011).

Inhalation

The chemical is classified as hazardous with the risk phrase 'Toxic by inhalation' (T; R23) in HSIS (Safe Work Australia). No data are available to evaluate this classification. An amendment to the classification (refer **Recommendation** section) is recommended, based on the following:

- observed methaemoglobinaemia following a single exposure (oral and i.p.) to relatively low doses of the chemical (see Acute toxicity:oral); and
- the chemical is absorbed by all routes of exposure.

Observation in humans

Secondary effects to methaemoglobin formation such as headache, dizziness, nausea and drowsiness have been reported in workers exposed to the chemical (EURAR, 2008; ACGIH, 2011).

Corrosion / Irritation

Skin Irritation

Limited data are available. The chemical was reported to slightly irritate skin in a study in New Zealand White rabbits conducted with a 50 % solution of the chemical in peanut oil. The effects were not sufficient to warrant a hazard classification (EURAR, 2008).

Eye Irritation

Limited data are available. The chemical was not considered to be an eye irritant in a study in New Zealand White rabbits. The irritation scores for each rabbit were not available (EU RAR, 2008).

Sensitisation

Skin Sensitisation

Limited data are available. The negative result observed for the chemical in a guinea pig maximisation test supports a conclusion that the chemical is not a skin sensitiser (EU RAR, 2008).

Repeated Dose Toxicity

Oral

The chemical is classified as hazardous with the risk phrase 'Danger of serious damage to health by prolonged exposure if swallowed (Xn; R48/22)' in HSIS (Safe Work Australia). The data available support this classification; in addition, given that the chemical is absorbed by all routes of exposure it is recommended that the classification should be applied to all routes (refer to **Recommendation** section).

Sub-acute and sub-chronic toxicity data are available for several species (rats, mice and dogs) (EU RAR, 2008; ATSDR, 2013). The target organs for toxicity appears to be the blood and male reproductive system (see **Reproductive and developmental** toxicity). Effects in the blood include:

- signs of hypoxia;
- decreased counts of haemoglobin and erythrocytes;
- increased counts of methaemoglobin, Heinz bodies and reticulocytes; and
- splenic haemosiderosis

In rats, based on haemosiderosis observed in the spleen of males in a 13-week study, the lowest observed adverse effect level (LOAEL) is 34 mg/kg bw/day. In dogs, a no observed adverse effect level (NOAEL) was established as 1 mg/kg bw/day on the basis of increased level of formation of methaemoglobin observed in a 13-week study.

In longer term studies (1–2 years), lesions in the kidneys and liver were observed in addition to effects reported above (EU RAR, 2008; ATSDR, 2013).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

The chemical is classified as hazardous—Category 3 mutagenic substance—with the risk phrase 'Possible risk of irreversible effects' (Xn; R68) in HSIS (Safe Work Australia). The available data support this classification.

The chemical is mutagenic in several strains of *Salmonella typhimurium* with and without metabolic activation. Mutagenic activity was increased in strains with elevated levels of both nitroreductase and O-acetyltransferase activities (YG1041 and YG1042) when compared to those observed in the parent strains (TA98 and TA100). The chemical was genotoxic in the *S. typhimurium* umu test with enhanced mutagenicity in strains with elevated levels of both nitroreductase and O-acetyltransferase activities.

Whilst consistent results have not been observed in vitro, the chemical is:

- mutagenic in a gene mutation assay with Chinese hamster ovary (CHO) cells incubated with rat liver S9 under anaerobic (reduced oxygen tension) conditions and in the P388 mouse lymphoma/TK system; and

- clastogenic in chromosomal aberration assays in human lymphocytes (without S9) and Chinese hamster lung (CHL) cells (with and without S9).

The chemical also caused morphological transformation in the Syrian hamster embryo (SHE) cells in the presence of activation and DNA damage (single-strand breaks) when tested at cytotoxic concentrations in the alkaline elution/rat hepatocyte assay.

Unscheduled DNA synthesis, micronuclei induction and DNA adduct formation have been observed in liver cells of rats treated with the chemical in vivo (EURAR, 2008; ACGIH, 2011; ATSDR, 2013). Chromosomal aberrations (chromatid breaks) have also been observed in lymphocytes and kidney cells (EURAR, 2008). DNA covalent binding was observed in several organs (liver, kidney, lung and mammary glands) of rats with the binding being highest in the liver. The covalent binding was decreased in the presence of sulfotransferase inhibitors, indicating that sulfation is important in the biotransformation of 2,4-DNT to reactive metabolites (EURAR, 2008). The chemical is not considered to cause dominant lethal mutations in rats and mice (EURAR, 2008).

Carcinogenicity

The chemical is currently classified as hazardous as a Category 2 carcinogen with the risk phrase ‘May cause cancer’ (T; R45) in HSIS (Safe Work Australia). The available data support this classification.

In long-term toxicity studies in rats, carcinogenic effects, including skin/subcutaneous tissue fibromas in males, mammary gland fibroadenomas in females and hepatocarcinomas in both sexes, were observed. In mice, kidney tumours (both benign and malignant) were reported to be significantly elevated in one long term study (EURAR, 2008). The purity of the test chemical was reported to range from 95–98 %. The test chemical, in some cases, was reported to contain the isomer 2,6-dinitrotoluene (2,6-DNT—CAS No. 606-20-2) (US EPA, 2002). No tumours were found in controls or in rats exposed to 2,4-DNT (99.9 % purity) at 27 mg/kg bw/day for one year (US EPA, 2003; ACGIH, 2011). The chemical demonstrated hepatocyte-foci promoting ability, but is less potent than its isomer 2,6-DNT (ACGIH, 2011).

The International Agency for Research on Cancer (IARC) overall evaluation is that the chemical is ‘possibly carcinogenic to humans’ (Group 2B) based on 'sufficient evidence in experimental animals' and 'inadequate evidence in humans' for the carcinogenicity of the chemical (IARC, 1996).

Reproductive and Developmental Toxicity

The chemical is classified as hazardous—Category 3 substance toxic to reproduction—with the risk phrase ‘Possible risk of impaired fertility’ (Xn; R62) in HSIS (Safe Work Australia). The available data support this classification.

Effects in the male reproductive system including decreased sperm production, reduced testicular weight, morphological alteration of Sertoli cells, and degenerated seminiferous tubules were observed in various studies in various species. A LOAEL of 0.57 mg/kg bw/day was established based on the atrophy of seminiferous tubules observed in a 24-month rat study (US EPA, 2002; EURAR, 2008; ACGIH, 2011; ATSDR, 2013). Effects on the ovaries (decrease of ovary weight and ovary atrophy) were observed in a study in mice with a NOAEL of 95 mg/kg bw/day.

In a three generation study with the chemical (98 % purity) in CD rats, reduced fertility was observed at the highest dose (34 mg/kg bw/day). A NOAEL of 3.9 mg/kg bw/day was established. No anomalies were detected in any of the offspring (US EPA, 2002; EURAR, 2008).

In a developmental toxicity study with Tg-DNT, pregnant rats were exposed orally for 14 days during gestation. Effects on foetal haematological parameters and organ weights were observed in the presence of maternal toxicity. Foetal malformations were not observed, even at dose levels that produced significant maternal toxicity. There was no effect on post natal development (OECD, 2004; ATSDR, 2013; IARC, 2013).

Reproductive effects such as an increased rate of spontaneous abortions and decreased sperm count have been observed in workers exposed to Tg-DNT. However, these studies have a number of limitations for evaluating adverse effects of the chemical in humans, such as exposure to other chemicals, small exposure populations in the studies and a lack of historical individual exposure monitoring (EURAR, 2008; ATSDR, 2013).

Other Health Effects

Neurotoxicity

Neurotoxic effects after intermediate or chronic duration exposure to the chemical were observed in a number of species, with symptoms ranging from tremors, convulsions, and ataxia to paralysis. Dogs appear to be most sensitive to the chemical with effects appearing to be cumulative. The NOAEL for male and female beagle dogs was 0.2 mg/kg bw/day of 2,4-DNT on the basis of neurotoxicity (incoordination and paralysis) derived from the 24-month study.

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic long-term effects (carcinogenicity, mutagenicity, reproductive toxicity) and systemic acute effects (acute toxicity by the oral/dermal/inhalation route of exposure). The chemical may also cause harmful effects following repeated exposure.

Public Risk Characterisation

Given the uses identified for the chemical, it is unlikely that the public will be exposed. Hence, the public risk from this chemical is not considered to be unreasonable.

Occupational Risk Characterisation

During product formulation, dermal, ocular and inhalation exposure of workers to the chemical may occur, particularly where manual or open processes are used. These may include transfer and blending activities, quality control analysis, and cleaning and maintenance of equipment. Worker exposure to the chemical at lower concentrations may also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical systemic long-term and systemic acute health effects, the chemical may pose an unreasonable risk to workers unless adequate control measures to minimise dermal and inhalation exposure to the chemical are implemented. The chemical should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine appropriate controls.

Guidance on the interpretation of workplace exposure standards for airborne contaminants advises that 'exposure to carcinogens should be eliminated or minimised so far as is reasonably practicable' (Safe Work Australia, 2013).

The data available support a minor amendment to the hazard classification in HSIS (refer to **Recommendation section**).

NICNAS Recommendation

Assessment of the chemical is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Currently an exposure standard exists in Australia for the commercial mixture of DNT (CAS No. 25321-14-6). The chemical is a primary mixture of this commercial mixture. The adequacy of the current exposure standard will be evaluated as part of the IMAP assessment for DNT.

Regulatory Control

Work Health and Safety

The chemical is recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical hazards and environmental hazards.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed (T; R39/23/24/25)	Causes damage to organs - Specific target organ tox, single exp Cat. 1 (H370)
Repeat Dose Toxicity	Harmful: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed (Xn; R48/20/21/22)	May cause damage to organs through prolonged or repeated exposure - Cat. 2 (H373)
Genotoxicity	Muta. Cat 3 - Possible risk of irreversible effects (Xn; R68)*	Suspected of causing genetic defects - Cat. 2 (H341)
Carcinogenicity	Carc. Cat 2 - May cause cancer (T; R45)*	May cause cancer - Cat. 1B (H350)
Reproductive and Developmental Toxicity	Repro. Cat 3 - Possible risk of impaired fertility (Xn; R62)*	Suspected of damaging fertility - Cat. 2 (H361f)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for industry

Control measures

Control measures to minimise the risk from oral/dermal/inhalation exposure to the chemical should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures which may minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;
- health monitoring for any worker who is at risk of exposure to the chemical if valid techniques are available to monitor the effect on the worker's health;

- air monitoring to ensure control measures in place are working effectively and continue to do so;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to assist with meeting obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((m)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemical are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (m)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals— Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemical has not been undertaken as part of this assessment.

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Last update 18 September 2014

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